

WHAT IS CLAIMED IS:

1 1. An isolated polypeptide having a strong formate dehydrogenase activity in the
2 presence of an organic solvent, said polypeptide comprising a mutation in which the cysteine
3 residue at position 146 or position 256 in the amino acid sequence of SEQ ID NO: 2 is
4 substituted with an amino acid other than cysteine.

1 2. The polypeptide of claim 1, wherein the substituted amino acid at position 146
2 is serine or valine.

1 3. The polypeptide of claim 1, wherein the substituted amino acid at position 256
2 is serine, alanine, or valine.

1 4. An isolated polypeptide having a strong formate dehydrogenase activity in the
2 presence of an organic solvent, in which the cysteine residues at positions 146 and 256 in the
3 amino acid sequence of SEQ ID NO: 2 are substituted with amino acids other than cysteine.

1 5. The polypeptide of claim 4, wherein the substituted amino acid at position
2 146 is serine or valine, and the substituted amino acid at position 256 is serine, alanine, or
3 valine.

1 6. The polypeptide of claim 1, wherein said polypeptide further comprises a
2 mutation in which the cysteine residue at position 6 in the amino acid sequence of SEQ ID
3 NO: 2 is substituted with an amino acid other than cysteine.

1 7. The polypeptide of claim 6, wherein the substituted amino acid at position 6 is
2 serine, alanine, or valine.

1 8. An isolated polypeptide selected from the group consisting of:
2 (1) the sequence of SEQ ID NO:2 in which cysteines at positions 6, 146, and 256
3 have been substituted with serine;
4 (2) the sequence of SEQ ID NO:2 in which cysteine at position 6 has been
5 substituted with alanine, and cysteine at position 256 has been substituted with serine;

- 6 (3) the sequence of SEQ ID NO:2 in which cysteine at position 6 has been
7 substituted with valine, and cysteine at position 256 has been substituted with serine;
- 8 (4) the sequence of SEQ ID NO:2 in which cysteine at position 6 has been
9 substituted with serine, and cysteine at position 256 has been substituted with alanine;
- 10 (5) the sequence of SEQ ID NO:2 in which cysteine at position 6 has been
11 substituted with serine, and cysteine at position 256 has been substituted with valine;
- 12 (6) the sequence of SEQ ID NO:2 in which cysteine at position 146 has been
13 substituted with serine;
- 14 (7) the sequence of SEQ ID NO:2 in which cysteine at position 256 has been
15 substituted with serine;
- 16 (8) the sequence of SEQ ID NO:2 in which cysteines at positions 146 and 256
17 have been substituted with serine;
- 18 (9) the sequence of SEQ ID NO:2 in which cysteine at position 256 has been
19 substituted with valine;
- 20 (10) the sequence of SEQ ID NO:2 in which cysteine at position 146 has been
21 substituted with serine, and cysteine at position 256 has been substituted with valine;
- 22 (11) the sequence of SEQ ID NO:2 in which cysteine at position 6 has been
23 substituted with alanine, and cysteine at position 256 has been substituted with valine;
- 24 (12) the sequence of SEQ ID NO:2 in which cysteine at position 6 has been
25 substituted with alanine, cysteine at position 146 has been substituted with serine, and
26 cysteine at position 256 has been substituted with valine; and
- 27 (13) the sequence of SEQ ID NO:2 in which cysteines at positions 6 and 146 have
28 been substituted with alanines, and cysteine at position 256 has been substituted with valine.

1 9. An isolated polynucleotide encoding the polypeptide of claim 1.

1 10. An isolated polynucleotide encoding the polypeptide of claim 8.

1 11. A vector into which the polynucleotide of claim 9 has been inserted.

1 12. A vector into which the polynucleotide of claim 10 has been inserted.

1 13. The vector of claim 11, wherein a polynucleotide encoding a reductase has
2 been further inserted into said vector.

1 14. The vector of claim 13, wherein said reductase is a carbonyl reductase derived
2 from *Kluyveromyces aestuarii*.

1 15. A transformant containing the vector of claim 11.

1 16. The transformant of claim 15, wherein the transformant is a microorganism.

1 17. A method for producing a polypeptide, said method comprising the step of
2 culturing the transformant of claim 15.

1 18. A method for producing (a) a polypeptide having a strong formate
2 dehydrogenase activity in the presence of an organic solvent, and (b) a reductase, said
3 method comprising the step of culturing a transformant containing the vector of claim 13.

1 19. The method of claim 18, wherein said reductase is a carbonyl reductase
2 derived from *Kluyveromyces aestuarii*.

1 20. A method for producing reduced form of β -nicotinamide adenine dinucleotide
2 from oxidized form of β -nicotinamide adenine dinucleotide, said method comprising the step
3 of contacting the polypeptide of claim 1 with oxidized form of β -nicotinamide adenine
4 dinucleotide.

1 21. A method for producing reduced form of β -nicotinamide adenine dinucleotide
2 from oxidized form of β -nicotinamide adenine dinucleotide, said method comprising the step
3 of contacting the transformant of claim 15 or a processed product thereof with oxidized form
4 of β -nicotinamide adenine dinucleotide.

1 22. A method for producing a reduced product from an oxidized substrate, said
2 method comprising the steps of:

3 (1) producing reduced form of β -nicotinamide adenine dinucleotide by the
4 method of claim 20; and

5 (2) recovering a reduced product generated by contacting the reduced form of β -
6 nicotinamide adenine dinucleotide of the step (1) and an oxidized substrate with a reductase
7 that produces the reduced product from the oxidized substrate in the presence of the reduced
8 form of β -nicotinamide adenine dinucleotide.

1 23. A method for producing a reduced product from an oxidized substrate, said
2 method comprising the steps of:

3 (1) producing reduced form of β -nicotinamide adenine dinucleotide by the
4 method of claim 21; and

5 (2) recovering a reduced product generated by contacting the reduced form of β -
6 nicotinamide adenine dinucleotide of the step (1) and an oxidized substrate with a reductase
7 that produces the reduced product from the oxidized substrate in the presence of the reduced
8 form of β -nicotinamide adenine dinucleotide.

1 24. The method of claim 22, wherein said oxidized substrate is a ketone and said
2 reduced product of the substrate is an alcohol.

1 25. The method of claim 23, wherein said oxidized substrate is a ketone and said
2 reduced product of the substrate is an alcohol.

1 26. The method of claim 24, wherein said ketone is 4-haloacetoacetate ester and
2 said alcohol is (S)-4-halo-3-hydroxybutyrate ester.

1 27. The method of claim 25, wherein said ketone is 4-haloacetoacetate ester and
2 said alcohol is (S)-4-halo-3-hydroxybutyrate ester.

1 28. The method of claim 22, wherein said reductase is *Kluyveromyces aestuarii*-
2 derived carbonyl reductase.

1 29. The method of claim 23, wherein said reductase is *Kluyveromyces aestuarii*-
2 derived carbonyl reductase.